Aquatic Botany 151 (2018) 30–37

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Reproduction of the mangrove species *Rhizophora stylosa* Griff. at its southern latitudinal limit

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ARTICLE INFO

Keywords:
Rhizophora stylosa
Mangrove
Latitudinal limit
Reproduction
Phenology
Climate change
Australia

ABSTRACT

A study of reproductive phenology and reproductive performance of the mangrove species *Rhizophora stylosa* was conducted in three estuaries spanning the southernmost 265 km of its known range on the east Australian coast. The timing of reproductive primordia, buds, flowers, fruit and propagule fall did not differ significantly over the latitudinal range of the study. Peak propagule fall occurred in April after a mean 19–20 months of development, only slightly longer than accounts for tropical latitudes. On average, 2.1% of buds matured to form viable propagules, with an average of 17.2% of buds becoming advanced fruit and 11.9% of advanced fruit becoming mature propagules, which compares well with the ranges published accounts for Australian tropical *R. stylosa* trees. However, there were relatively few buds per inflorescence on recorded shoots in this study, although formal studies on this in the tropics are absent. Within-site variation in bud and propagule production per shoot was greater than variability between sites in many cases, though our sample size was necessarily low due to the rarity of the trees at the southern limit and the detailed recording method. The species reproduces successfully at its known southern limit, with notable comparability to the eastern Australian Tropics. This suggests there is little thermal limitation on reproduction at the southern limit, where there is evidence of slow spread. "Precocious" reproduction as observed for young *R. mangle* at range limits in North America was not detected.

1. Introduction

Climate has long been implicated inthe latitudinal range of mangrove species, principally through the "climate sifting" of species on cold tolerance (Hutchings and Saenger, 1987; Duke et al., 1998). Recent poleward expansion of mangrove limits has been attributed in part to recent global warming (e.g. Saintilan et al., 2014; Alongi, 2015). However, the response may not be as simple as a step-wise extension of latitudinal range (Alongi, 2015). For example, temperature thresholds did not consistently predict mangrove species limits in the global studies of Quisthoudt et al. (2012) and Osland et al. (2017).

There is no *a priori* reason why the responses of phylogenetically disparate mangrove taxa should be the same, or be independent of the climatic, hydrographic and geomorphic features of different coasts. Since the last major freeze event in 1989, the limits of *Rhizophora mangle* L. have rapidly extended poleward on the US Atlantic coast (Cavanaugh et al., 2014) and the species is reaching sexual maturity early there ("precocious" reproduction) (Dangremond and Feller, 2016). Congeneric case studies can shed light on how typical not only range extensions are, but also identify any other such "anomalous" responses.

Rhizophora stylosa Griff. is a widespread Indo-Pacific mangrove species, ranging from Okinawa Island in the north, to Fiji and the Marshall Islands in the east and to Odisha State, India in the west (Duke, 2006; Kamruzzaman et al., 2013; Ragavan et al., 2016). Its known southern limit is the estuary of South West Rocks Creek (SWR Creek; -30.88°, 153.03°) on the east Australian state of New South Wales (NSW) (Wilson, 2009; Table 1). It occurs in relatively large numbers on the northernmost NSW estuary of the Tweed River (-28.18°, 153.53°), albeit in far smaller numbers than in the Australian tropics (Duke, 2006). South of the Tweed River, it was recorded in lower numbers in 15 of 26 estuaries searched south to the Hastings River (−31.43°, 152.92°) by Wilson (2009). No new estuary records have since been confirmed, although survey effort has only been slight.

Rhizophora stylosa shows evidence of recent expansion on intra- and extra-estuary scales in NSW, but clearly long-established stands are present in about nine estuaries (Wilson, 2009). NSW has a high-energy coast and mangroves are effectively restricted to estuaries.

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https://doi.org/10.1016/j.aquabot.2018.07.009
Received 6 April 2018; Received in revised form 11 July 2018; Accepted 21 July 2018
Available online 25 July 2018
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Table 1
Details and notes on the estuaries and trees sampled. Estuary details: L = Location; W = Water area; C = Catchment area; M = Mapped area of mangrove (Creese et al., 2009); RS: Known number of *R. stylosa* plants producing buds / producing propagules for the whole estuary in 2004 (Wilson, 2009). Water and catchment areas from http://www.environment.nsw.gov.au/estuaries/list.htm.

Estuary	Estuary details	Number and name code of sampled trees and notes
Brunswick River	L: -28.54°; 153.56° W: 3.6 km^2 C: 226.3 km^2 M: 123.3 ha RS: 120 / circa 35	The Brunswick River system includes 3 arms, including a northern arm known as Marshalls Creek. 7 trees were sampled in the two loose main stands on Marshalls Creek, plus 1 isolated tree on the main Brunswick River arm. Site analyses are restricted to the 7 Marshalls Creek trees.
Richmond River	L: -28.88°; 153.59° W: 38.4 km^2 C: 6861.8 km^2 M: 602.6 ha RS: 84 / circa 44	4 trees: 3 trees were sampled from the only stand at Mobbs Bay, and a single isolated tree on the South Ballina shoreline. The Mobbs Bay stand is longer established than those on Marshalls Creek, as it is denser and with bigger trees.
South West Rocks Creek	L: -30.88°; 153.04° W: 0.9 km^2 C: 3.7 km^2 M: 64.8 ha RS: 3 / 2	2 propagule-producing trees were present only. One more produced buds only during the study, but propagules were recorded in January 2016.

Rhizophora has an unusual anatomy and physiology (Tomlinson, 1994). Reproductive buds arise within the meristematic apical bud of each shoot and paired reproductive bud primordia (hereafter: primordia) sit in the axils of the two new leaves after bud expansion (Tomlinson et al., 1979; Tomlinson, 1994). Shedding of the stipules on bud expansion leaves annular stipular scars ("nodes") that can be used as reliable reference points for the tracking of vegetative and reproductive dynamics on the shoot. As a shoot extends with new leaf emergences above a given locus, new overlapping reproductive generations may develop (Supplementary Fig. S1).

The primordia develop into dichotomised inflorescences, with a variable number of divisions determining the number of buds within and between *Rhizophora* species (Duke and Bunt, 1979). Fertilised flowers develop slowly into acorn-sized fruit, which germinate and develop on the tree into viviparous seedlings ("propagules") (Supplementary Fig. S2). The involved vivipary (Juncosa, 1982) means a lengthy reproductive cycle in *Rhizophora*, with a reported range of 8–13 months in *R. mangle* (Gill and Tomlinson, 1971) to "nearly 3 years" in *Rhizophora apiculata* BL. (Christensen and Wium-Andersen, 1977). A low rate of conversion of buds and/or flowers to propagules has been found to be typical of *Rhizophora* (e.g. Saenger, 2002; Tyagi, 2004; Coupland et al., 2006).

Seasonal peaks in reproductive phenophases are consistently observed for *R. stylosa* (Duke et al., 1984; Leach and Burgin, 1985; Duke, 2002; Coupland et al., 2005; Kamruzzaman et al., 2013) and other *Rhizophora* spp. (e.g. Steinke, 1988; Duke, 2002; Mehlig, 2006; de Lima Nadia et al., 2012). Seasonal timing of reproductive phenophases is not always strictly in accordance. For example, the short flowering stage of *Rhizophora* spp. has been observed during drier seasons (e.g. Duke et al., 1984) and during wetter seasons (e.g. Fernandes, 1999; Mehlig, 2006). Although methodologies and assumptions can differ between studies, often qualified comparisons can be made.

Inter-annual variation in propagule production has been widely observed in *Rhizophora* (Gill and Tomlinson, 1971; Duke et al., 1984; Duke, 2002; Coupland et al., 2005; Mehlig, 2006; Kamruzzaman et al., 2013). Tyagi (2003, 2004) found more flowers and a greater number and size of propagules in *R. stylosa* in "wet" versus "dry" sites in Fiji, suggesting local conditions may be significant.

This study examines the reproduction of *R. stylosa* at its southern limits, partly in the context of warming over recent decades. The data are from the same shoots at the same time as the vegetative study of Wilson and Saintilan (2012) and the two studies are best considered together. Wilson and Saintilan (2012) found no latitudinal clines in vegetative phenology or leaf initiation over the study area and no evidence for a thermal growth limit at SWR Creek. The results were generally comparable to tropical studies.

Effectively all reproduction in *R. stylosa* is sexual and the metabolic costs of reproduction can be considerable (Bazzaz et al., 2000), which might be hypothesised to be more significant at poleward limits. Recent "precocious" reproduction in *R. mangle* (Dangremond and Feller, 2016) suggests potential for anomalous reproductive efforts at the poleward limit as conditions change.

Specific aims of this study were firstly to characterise *R. stylosa*'s reproductive phenology at its southern limits, including propagule maturation time. Secondly, to determine quantitative reproductive success in propagules produced, and the rate of conversion of buds and fruit to propagules.

Null hypotheses are no differences in maturation times and rates of conversion between latitudes in NSW, or between the pooled NSW data and published tropical Australian studies as far as comparisons allow.

2. Methods

2.1. Background and sample selection

Much background in Wilson and Saintilan (2012) applies and so is not repeated in detail here. The study area has a sub-tropical climate, with warmer than average conditions during the study, including over winter. All monthly maxima were higher at SWR Rocks (Smoky Cape Lighthouse station; data from 1939) than the long-term mean by 0.8 to 1.5 °C (+ 1.3 °C overall). Monthly minima were at or above the long term mean, including in the coldest month of July (+ 0.2 °C overall; Wilson, 2009). Rainfall was lower than the long-term means for most months, with major annual deficits of 14% in 2003 and 21% in 2004 against the long-term mean (data: BOM, 2018a).

Selected shoots on 14 mature trees in three estuaries over 265 km of latitude (Table 1) were intensively and repeatedly sampled from October 2002 until October 2004. The sampled trees are the "mature" trees of Wilson and Saintilan (2012) except one isolated slow-growing tree in the Brunswick River estuary only just starting to produce buds. Site analyses for the Brunswick River system only include trees sampled on the Marshalls Creek arm (Table 1).

A range of shoot sizes around the circumference of each tree were selected. The commonplace litterfall trapping is unsuitable for frequently isolated trees of differing (usually small) canopy size, and shoot tracking is well-suited to *Rhizophora* anatomy and information rich, albeit labour intensive. The inter-sampling period averaged 73 days on the Richmond River and SWR Creek and 72 days on the Brunswick River system. Two mature trees only at SWR Creek is not ideal, but the next comparable estuary is 95 km north at Corindi Creek (−30.02; 153.13), pre-judged as too distant from the southern limit. Austral seasons are referred to unless noted.

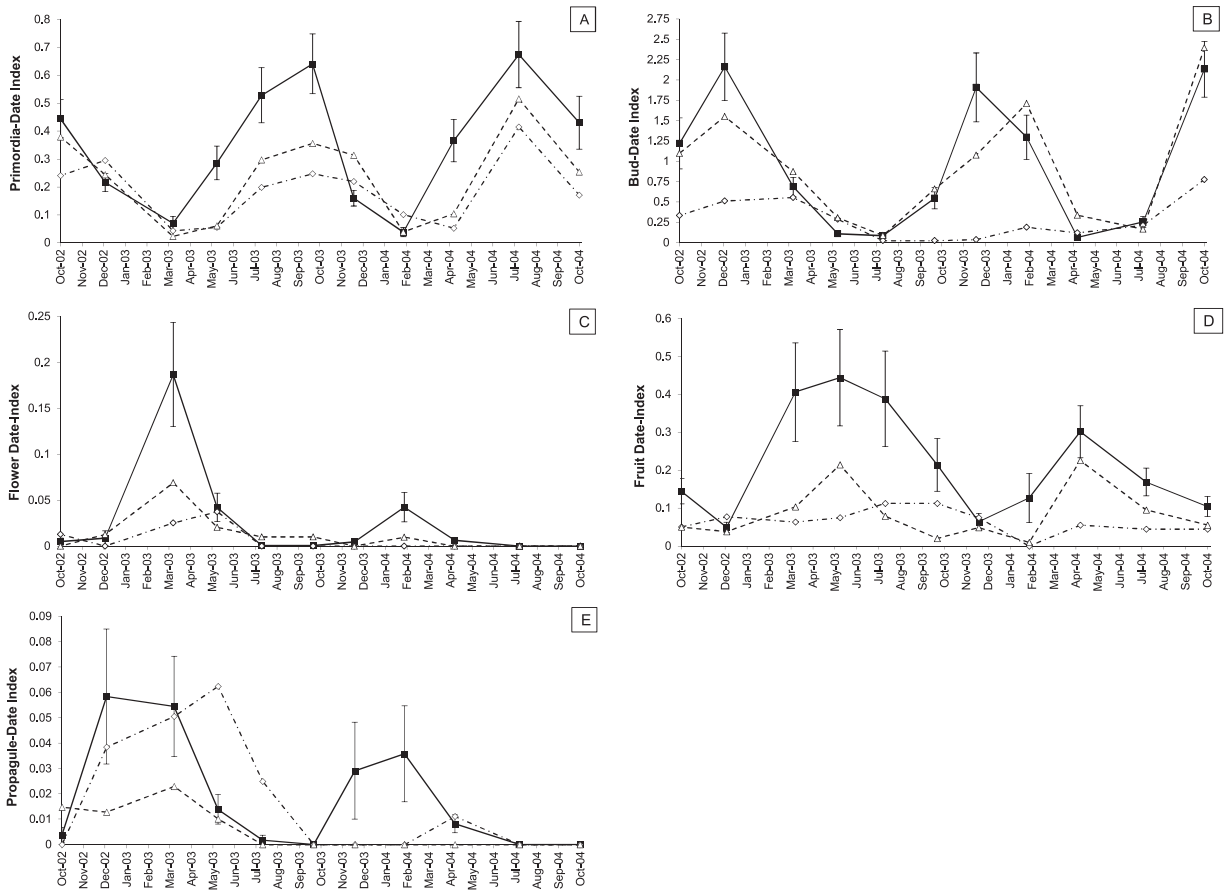

Fig. 1. Plots of the [Organ]-Date Indices for: (A) flower primordia; (B) flower buds; (C) open flowers; (D) advanced fruit; and (E) propagules for 14 sampled trees (closed square), plus data from two trees at South West Rocks, SWR1 (open diamond) and SWR5 (open triangle). The [Organ]-Date Indices are the tree means of each tracked organ stage on each date, divided by the number of sampled shoots on that date (see text). Error bars are the standard error of the mean.

2.2. Sampling and data recording techniques

Basic data were repeated counts of defined reproductive organs on tracked shoots, including initially marked shoots and any side shoots formed by the branching on existing tracked shoots during the study. The new shoots may produce reproductive organs (after a period) and are important in the "modular" vegetatively expansion of relatively small tree crowns as was common in this study. A mean of 12.85 (± 6.62) shoots were initially sampled per tree, with higher numbers of shoots sampled on larger trees and on the two trees at SWR Creek.

Six categories of reproductive organs were recorded (Supplementary Figs. S1 and S2):

- Flower bud primordia: Formed in pairs with new leaf pairs.
- Buds: Recorded from the time the number of buds in an inflorescence could be determined from a developing primordium, even if they are not fully developed.
- Open flowers: A short stage where petals and stamens are still present before falling.
- Fruit: Fruit were counted if organs were being retained after petal fall, even before swelling of the ovary wall. Well-developed fruit with a diameter > 8 mm were distinguished as "advanced fruit". All "fruit" are used to illustrate phenology, but "advanced fruit" are used in the statistical analysis.
- Propagules: Recorded from the time the hypocotyl first erupted from the base of the fruit, even though considerable development was still needed.

Tree height and diameter ($D_{1.3}$, or just above any prop roots at 1.3 m) were recorded at the end of sampling.

2.3. Data compilation and analysis

The sampling period was not fixed for all shoots, as new sampled shoots were added at different times and rates during the study. This precludes many simple measures; hence, two indices were devised and applied in turn to the sampled stages.

Firstly, four indices per tree of the means of the number of Primordia, Buds, Fruit and Propagules on all sampled shoots on each sampling date, divided by the total number of shoots sampled per tree on that sampling date, called [Organ]-Date Indices, e.g. Bud-Date Index. There are indices for each sampling date and they are used to illustrate reproductive phenology and to compare relative organ production temporally during the study between trees and sites. The divisor includes all tracked shoots even if not reproductive on the sampling date.

Secondly, four indices of the tree sums over the whole study period of, in turn, Primordia, Buds, Fruit and Propagules divided by the number of originally marked shoots on each tree, called "[Organ]-Original Shoot Indices', e.g. Bud-Original Shoot Index (Bud-OSI), plus one index using the sums of all four stages (Total-OSI). One of each OSI per tree is generated allowing comparison of tree-scale relative reproductive organ production. Dividing by the number of original shoots gives a common reference, accounting for differing numbers of shoots originally marked on a tree and any organs produced on branched shoots during the study.

Other shoot level data, such as the proportion of reproductive shoots and the number of buds per inflorescence, along with "reproductive

Table 2
Site totals for bud, fruit and propagule numbers for the 2002-04 reproductive generation and propagule totals for the 2001–2003 generation (recorded once off in late 2003), plus site mean % conversion of buds and advanced (adv.) fruit to propagules on tracked shoots. Mean count of flower buds per inflorescence on three dates near peak bud occurrence in three years (n = 1405 inflorescences) is also shown. All trees are included in the data. Individual tree data in Supplementary Table S1.

	2003	2002-2004 generation						
Estuary	Propagules	Buds	Advanced Fruit	% Buds to adv. fruit	% Buds to Propagules	% Adv. Fruit to Propagules	Propagules	Mean no. buds per inflorescence
Marshalls Creek	27	1053	208	19.75	2.56	12.98	27	2.87 ± 0.18SE
Richmond River	5	313	63	20.13	1.60	7.94	5	2.71 ± 0.19SE
SWR Creek	7	258	14	5.43	0.78	14.29	2	2.52 ± 0.17SE
Total	**43**	**1652**	**285**	**17.25**	**2.06**	**11.93**	**34**	**2.72 ± 0.14SE**

success" measures of the percentage conversion of buds and fruit to propagules, were recorded or derived from the data.

The raw bud data are normally distributed, but the raw fruit and raw propagule data are not as determined by Anderson-Darling tests, so for consistency non-parametric tests were undertaken using R 2.14.0 software (R Core Team, 2014). Correlations used the Kendall's τ-b statistic (McLeod, 2015) using data from the originally marked shoots only and other parameters recorded during this study. Kruskal-Wallis analysis with post-hoc pair-wise comparison by Wilcoxon rank sum tests with Holm's sequential Bonferroni correction (Yatani, 2014) was used to test many differences, including in the Organ-Date and Original Shoot indices. Significance is taken to be < 0.05.

3. Results

3.1. Reproductive phenology

All trees exhibited peak seasonality in reproductive phenophases, although some primordia and buds formed all year (Fig. 1A-E; data for the two mature trees at SWR Rocks are included for comparison to the whole data set). The usual process was: primordia appear in winter/spring with the single cool season leaf initiation (peak circa July-September; Fig. 1A), followed by the progressive development of buds (Fig. 1B); flowers open briefly the following autumn (peak circa March; Fig. 1C); fruit mature until early summer (peak circa late November-December; Fig. 1D) when the radicle erupts from the fruit and the propagules mature before dropping in autumn (peak in April; Fig. 1E).

Flower buds peaked in mid-summer (Fig. 1B). The steep bud loss from the peak commenced first through abortion, which was common but unquantified, then subsequently by flower opening. Bud number was lowest in April-May (Fig. 1B). Flowering was strongly congruent, although small numbers of flowers opened slightly outside the main period of February-March (Fig. 1C).

Broad peaks in fruit number reflect the length of this phase (Fig. 1D). The relatively steep fall from circa July-August to circa early December was due to widespread fruit failure, which tended to accelerate later in the stage. The propagule phase from the eruption of the radicle from the fruit in early summer to propagule fall was typically about five months, and the broad peaks reflect this maturation time (Fig. 1E). The sharp drop in March/April represents peak propagule fall, although the earliest fall was observed at the end of February and the latest in July. Loss after the radicle had emerged, but before maturity, was very rare.

The typical length of the *R. stylosa* reproductive cycle in NSW was estimated from the phenological data as 19–20 months, as primordia emergence peaked in August/September and propagule fall peaked in April of the second year. The happenstance of the field study commencement (October) and the long cycle meant full tracking of a complete cycle over all shoots was impossible. However, maturation times shorter than the norm were fully tracked; while 76% of primordia resulting in propagules in 2004 were present in October 2002, 9% emerged as late as May 2003.

3.2. Quantitative comparisons and reproductive success

3.2.1. Reproductive production

The mean percentage of reproductive shoots to all shoots on all trees over the sampling dates was 44.5% ± 1.71SE (range: 20.3%–77.9%). The mean number of all post-primordium reproductive organs per reproductive shoot per sampling date was 2.40 ± 0.13SE (range: 1.50–3.89). There was no correlation between the production of all reproductive organs on reproductive shoots and the rate of leaf initiation on those shoots (τ-b = -0.13; P = 0.54), nor with tree height (τ-b = -0.02, P = 0.96) or $D_{1.3}$ at the end of the study on single stemmed trees (τ-b = -0.16, P = 0.59).

Intact inflorescences most commonly contained 2 buds (range: 1–8). Based on counts on three dates in three years near the peak of bud presence on all trees, the mean number of buds per inflorescence was 2.72 (range: 1.82–3.92) (Table 2). There was significant variation between trees as a group (H = 25.54; DF = 13; P = 0.02), but between-site variation was not significant (H = 2.79; DF = 2; P = 0.24), despite an apparent fall with increasing latitude (Table 2). Only one tree at SWR Creek was particularly low. Usually one pair of inflorescences was present on a shoot, but two pairs were present on circa 25% of these sampled shoots and three pairs in < 0.5% of samples.

3.2.2. [Organ]-date indices

There was significant variability in the Primordia-Date Index within all trees as a group (Table 3), although there were no significant pair-wise differences between individual trees. SWR Creek was significantly

Table 3
Ranges and Kruskal-Wallis analysis of variance (H value) for Original Shoot Indices (OSI) and [Organ]-Date Indices for reproductive phases. OSI values are between sites (estuaries) (df = 2). The Tree [Organ]-Date Indices include all trees as a group (df = 13) and the Site [Organ]-Date Index includes three sites as a group (df = 2).

	Site OSI			Tree [Organ]-Date Index			Site [Organ]-Date Index	
Organ	Mean ± SE	Range	H (P value)	Mean ± SE	Range	H (P value)	Range	H (P value)
Primordia	4.76 ± 0.68	1.56 - 9.63	3.45 (0.18)	0.34 ± 0.03	0 - 1.75	37.29 (**< 0.01**)	0.19 - 0.45	7.85 (**0.02**)
Buds	12.67 ± 2.00	3.26 - 25.79	1.16 (0.56)	0.95 ± 0.32	0 - 6.10	24.39 (**0.03**)	0.99 - 1.22	1.61 (0.45)
Fruit	2.97 ± 0.68	0.45 - 8.60	2.51 (0.28)	0.22 ± 0.08	0 - 1.71	45.13 (**< 0.01**)	0.08 - 0.27	7.04 (**0.03**)
Propagules	0.25 ± 0.09	0 - 0.47	0.55 (0.76)	0.19 ± 0.18	0 - 0.35	31.78 (**< 0.01**)	0.01 - 0.03	1.93 (0.38)
Total	16.24 ± 2.64	3.95 - 33.19	0.79 (0.67)	–	–	–	–	–

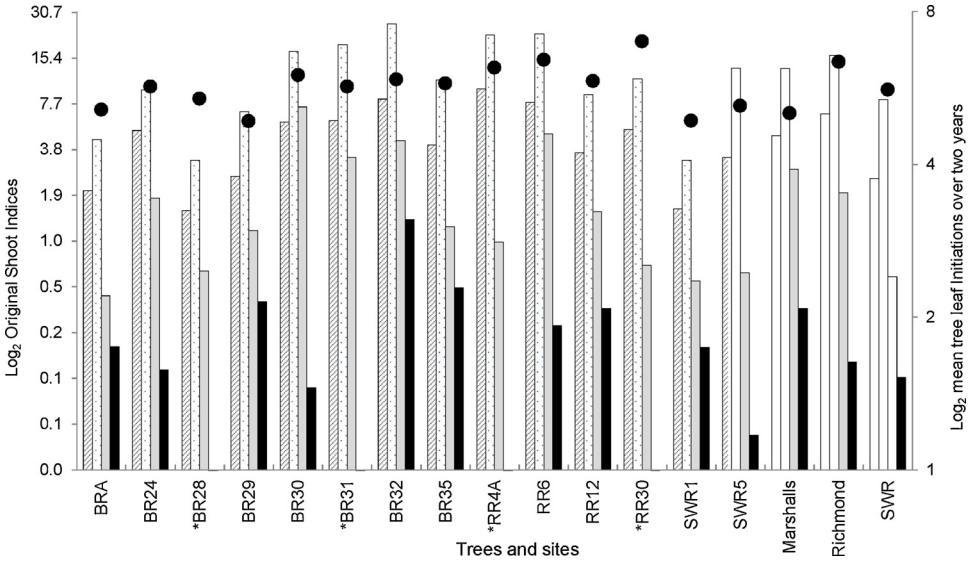

Fig. 2. Log_2 plot of values of Original Shoot Indices for (left to right): flower primordia (cross hatch); flower buds (dots); advanced fruit (solid grey); and propagules (solid black) for sampled trees and three sites. Original Shoot Indices are the sums over all sampling dates of each reproductive organ stage on sampled shoots per tree, divided by the number of originally marked branches on the tree (see text). Four trees marked with asterisks did not produce propagules on tracked branches. Filled circles are Log_2 tree mean leaf initiations on sampled shoots for the trees and sites over two years (Wilson and Saintilan, 2012). For more detail, see Section 3.2.

lower as a site than Richmond River (P = 0.02), but not quite from Marshalls Creek (P = 0.09). Richmond River and Marshalls Creek did not vary significantly (P = 0.17). The Bud-Date Index varied significantly within all trees as a group, but between-site variation was not statistically significant (Table 3). SWR Creek was the lowest ranked site, although some individual trees elsewhere were indistinguishable.

The Fruit-Date Index varied significantly within all trees as a group and between sites (Table 3). SWR Creek is significantly lower than the other sites, but the Richmond River and Marshalls Creek did not differ significantly (Table 3). The only significant pair-wise differences were one tree at SWR Creek with two trees at Marshalls Creek and one at the Richmond River. The Propagule-Date Index varied significantly within all trees as a group, but not between sites (Table 3). Unexpectedly, one tree on Marshalls Creek strongly dominated propagule numbers with 44.1% and 39.5% of all propagules on sampled shoots in the 2001–2003 and 2002–2004 generations (Supplementary Table S1).

3.2.3. Original shoot indices (OSI)

The OSIs show varied reproductive organ product over the study between trees, some nearby each other (Fig. 2). On a site basis, SWR Creek was the lowest for Primordia, Bud and Fruit-OSI, but similar to the Richmond River on the Propagule-OSI (Fig. 2; Table 3). None of the site OSIs differed significantly (Table 3).

Based on data from all originally marked shoots, significant correlations were found between Primordia-, Bud- and Fruit-OSI, but not between the Propagule-OSI and any earlier stage (Table 4). Moderate, but significant, correlations were found between tree mean leaf initiations (Wilson and Saintilan, 2012) and the Primordia-, Bud-, Fruit- and Total-OSIs, but again not the Propagule-OSI (Table 4). There were no significant correlations of any OSI with tree height or trunk diameter ($D_{1.3}$ on single stemmed trees) at the end of the study (Table 4).

3.2.4. Conversion rates and propagule production

The rates of conversion of buds to advanced fruit, buds to propagules and advanced fruit to propagules was inconsistent between trees and sites (Table 2; tree data in Supplementary Table S1). Mean bud to propagule conversion did not differ significantly between sites (H = 1.29, DF = 2, P = 0.52), despite varying from north to south.

There were more propagules on originally marked shoots in the 2001–2003 reproductive cycle than for the tracked 2002–2004 cycle (Table 2; Supplementary Table S1), due to fewer propagules at SWR Creek. Totals at Marshalls Creek and the Richmond River were identical in both years, but not necessarily on the same trees. The difference between years was not significant (Wilcoxon rank sum test W = 119, DF = 1, P = 0.33). If trees with no propagule production on tracked shoots are removed, there is only a faint indication of alternating propagule generations.

Table 4
Rank correlation coefficients (Kendall's τ-b) and P values (parentheses) between the cumulative number of all primordia, buds, fruit, propagules and the Original Shoot Indices (OSI), plus correlation coefficients for the OSIs with leaf initiation rate (Wilson and Saintilan, 2012) and tree height and $D_{1.3}$ (single stemmed trees only) at the end of the study. See Section 3.2.

	Primordia-OSI	Bud-OSI	Fruit-OSI	Propagule-OSI	Total-OSI
Primordia-OSI	1	–	–	–	–
Bud-OSI	0.85 (< **0.01**)	1	–	–	–
Fruit-OSI	0.65 (< **0.01**)	0.63 (**0.02**)	1	–	–
Propagule-OSI	0.01 (1.00)	0.01 (1.00)	0.10 (0.66)	1	–
Leaf initiation	0.55 (< **0.01**)	0.49 (**0.02**)	0.42 (**0.04**)	−0.14 (0.54)	0.46 (**0.03**)
Tree height	0.09 (0.70)	0.05 (0.87)	0.16 (0.48)	0.12 (0.61)	0.09 (0.7)
$D_{1.3}$	0.22 (0.47)	0.33 (0.25)	0.22 (0.47)	0.09 (0.83)	0.39 (0.18)

4. Discussion

4.1. Phenology and length of the reproductive cycle

Phenological phases were consistent across the study area, especially in the relatively tight peaks of buds and flowers, which supports the null hypothesis of no difference, albeit with some variability within the seasonal patterns. By contrast, Clarke and Myerscough (1991) found flowering initiation in the dominant mangrove species, *A. marina*, varied by 2–3 weeks over 200 km of the central NSW coast. Wan Juliana et al. (2011) report quite different lengths of their "bud season" for *R. stylosa* at two relatively nearby sites in Malaysia and Wium-Andersen (1981) found propagule fall varied by two months for *R. mucronata* over 60 km in southern Thailand.

Beginning phenological observations at the primordium stage is rare; however, *R. stylosa* primordia peaked in August at Hinchinbrook Island (Duke et al., 1984; -18.36°, 146.15°), similar to this study. Using litterfall, Duke et al. (1984) found maximum bud fall in summer, apparently comparable to this study as ongoing bud failure during the summer bud peak was high in NSW.

Using direct shoot data, Coupland et al. (2005)'s very broad bud peak at Darwin Harbour (-12.50°, 130.83°) was in early summer, but their lowest bud numbers were in March versus May-June in NSW. Their bud (and fruit) peaks are broad as they report the percentage of sampled shoots with buds, which does not account for differing numbers of buds per inflorescence, the loss of some buds, or multiple inflorescences on a shoot. Hence, patterns are less pronounced than in shoot tracking or litterfall data.

Peak *R. stylosa* flowering in northern NSW (February-March) was comparable to north Queensland (February-March; Duke, 2002) and PNG (January-March; Leach and Burgin, 1985) and a little earlier than Fiji (March-June; Tyagi, 2004). By contrast, Coupland et al. (2005) reported peak flowering in December/January at Darwin Harbour.

The peak fall of "mature fruit" in September in north Queensland (Duke et al., 1984) is comparable to the period of high failure of advanced fruit in NSW (July-September). In the only other extra-tropical *R. stylosa* reproductive study on Okinawa, peak flowering and bud fall were in comparable seasons to NSW, but peak fruit fall appeared to be in the opposite season (Kamruzzaman et al., 2013).

Peak *R. stylosa* propagule fall was reported as February-March in north Queensland generally (Duke, 2002) and at Dunk Island (Banfield 1908; -17.95°, 146.16°) and Port Curtis (Saenger, 1982; -23.92°, 151.42°) specifically. Peak propagule fall in NSW was slightly later (April), although viable propagules fell as early as late February. In contrast to NSW and north Queensland, Coupland et al. (2005) report peak propagule fall in Darwin Harbour during the mid-year dry season. Tyagi (2004) implies propagule drop is in March in Fiji and Kamruzzaman et al. (2013) report peak propagule fall on Okinawa in a similar season (August-September) to NSW.

Coupland et al. (2005) suggest "different monsoonal regimes" may be responsible for phenological differences between Darwin Harbour and north Queensland, but climatic differences are much greater between tropical Queensland and NSW. Northern NSW is less seasonal than the tropics, but February-April is the wettest period. Duke et al. (1984) suggest dispersal advantages from high river flow during wet seasons, and most mangrove species produce disseminules during the local wet season (Saenger, 2002).

The typical time from primordia to propagule fall in *R. stylosa* in NSW (circa 19–20 months) was slightly longer than the 16–20 months at Hinchinbrook Island (Duke et al., 1984). However, some shorter cycles to meet the seasonal propagule fall were recorded in this study. The typical time from flowering to propagule fall was 12–13 months, compared to 11 months in Darwin Harbour (Coupland et al., 2005) and 11–12 months in north Queensland (Duke, 2002).

The dominant *Avicennia marina* subsp. *australasica* (Walp.) J.Everett extends much further south than *R. stylosa* (to -38.75°), but experiences more dramatic latitudinal impact. Duke (1990, 2002) recorded flowering six months later and an increase in flower to fruit maturation time from 2 to 3 months to 10 months in the most southern populations of *A. marina* compared to the tropics. Any difference in maturation times between *R. stylosa* in NSW and those published for the tropics appeared small with some overlap, hence a null hypothesis of no difference between NSW and the tropics cannot be rejected. Arguably there is little import to any slightly longer maturation times due to resource restriction or other factor if the propagules fall within the same season. Kamruzzaman et al. (2013) recorded 9–10 months from flowers to propagule fall ("mature propagules") on Okinawa, interestingly shorter than in NSW and tropical Australia.

4.2. Quantitative comparisons and reproductive success

4.2.1. Organ production

The OSIs show the sum production over the study of each phase of the reproductive cycle varied between trees (e.g. observable in Fig. 2). However, there were very few significant pairwise comparisons using Wilcoxon rank sum tests and no significant site differences in any OSI were detected, suggesting intra-estuary variability was stronger than inter-site differences such as latitude. Results may be inconsistent; for example, tracked shoots at the Richmond River produced more buds but fewer fruit than those at Marshalls Creek. Sample sizes are low and propagule analyses in particular may be weakened by the low numbers (34 propagules from 1652 buds), although that is a result in itself.

All the tree [Organ]-Date Indices indicated significant inter-tree variation in primordia, fruit, bud and propagule production over the sampling dates, but only the Primordia- and Fruit-Date Indices varied significantly on a site basis. As interim stages there is no material impact on fecundity if the differences do not carry through to propagule production.

The correlations between Primordia-, Bud- and Fruit-OSI were expected, however no earlier stage correlated with the propagule stage, contradicting an intuitive idea that more buds and fruit set should mean more propagules. Loss during the fruiting stage was most responsible. Few, if any, factors predicting propagule production were detected, including no correlation with tree size and shoot growth rate. Even nearby trees with putatively similar environmental variables such as solar access and sediment differed in fecundity. Controlling factors remain obscure, as do reasons for the dominant performance of one tree (BR32), which extends beyond the tracked shoots, and was also observed in 2010 and 2016.

Inter-annual variation was not statistically significant on tracked shoots, partly due to the influence of trees with zero propagule production (Supplementary Table S1) but was observed consistent with the literature. For example, in an isolated stand on the Brunswick River, there were < 10 propagules in 2003, circa 100 in 2004 and zero in January 2016, although such differences are not as dramatic in bigger, more fruitful stands. One tree at SWR Creek (SWR5) branched relatively strongly in 2004, producing no propagules in that year compared to 8 in 2002, 13 in 2003 and 9 in 2005, conceivably because branches arise in the same axillary space as reproductive primordia. Many *A. marina* trees do not reproduce every year in NSW (Clarke and Myerscough, 1991). We thus caution against comparisons made on single year observations.

4.2.2. Bud conversion

The general finding that conversion of buds and flowers to propagules is low in *Rhizophora* is supported, perhaps due to combinations of genetic factors, inefficient pollination and prevailing conditions. Regardless, only *Avicennia* rivals *Rhizophora* for dominance as a global mangrove genus and apparently inefficient reproduction has not been a barrier to biogeographic success. The overlapping reproductive generations may be a factor; in this study, the rise in new buds mirrored the fall in existing fruit, with a simple correlation of $r = -0.81$, and maybe resource limitation, perhaps mediated by prevailing conditions, is operative.

The rates of conversion through the cycle in this study compare well with tropical studies. The bud stage is the earliest for meaningful comparison, although not often assessed. However, Coupland et al. (2006) recorded 0.5% of buds progressing to propagules in Darwin Harbour, much lower than in this study (2.1%). Bud conversion to advanced fruit of 17.2% in NSW was much higher than the 3.0% conversion to young fruit in Darwin Harbour (Coupland et al., 2006) and estimates of 7.2% for *R. mangle* and 13.0% for *R. apiculata* (Hogarth, 1999).

However, conversion of fruit to propagules was lower in this study (11.9%) than the 25% recorded by Coupland et al. (2006), although this only partially compensates for the lower bud to fruit conversion in Darwin Harbour. The greatest loss in Darwin Harbour appeared to be as buds/flowers, compared to fruit in NSW. This is unexplained, and more effective pollination in the sparse stands and isolated trees in NSW over tropical stands seems unlikely. Kamruzzaman et al. (2013) found 6.7% conversion from fruit to propagules in *R. stylosa* on Okinawa, lower than the current study, although one of their years was comparable

(11.4%).

The high bud conversion rate in NSW does not necessarily denote greater fecundity, however. Although bud number did not correlate with propagule production on a shoot scale in this study, hypothetically trees with more buds in total due to more reproductive branches, more inflorescences per branch and/or more buds per inflorescence may produce more propagules even if conversion rates are lower. Tropical data for comparison to this study are lacking, but, for example, the mean bud number per inflorescence in this study (2.7) was lower than 4–8 buds cited for Queensland (Duke, 2006; range: 1–64) and Malaysia (Mohd Nasir and Safiah Yusmah, 2007).

Proffitt and Travis (2014) found increased reproductive potential with latitude in recently expanded *R. mangle* range, but this cannot be categorically determined for *R. stylosa* despite apparently high conversion rates. There was no evidence of a latitudinal effect over the study area and a hypothesis of no increased fecundity in *R. stylosa* at the southern limits remains. Kamruzzaman et al. (2013) concluded propagule litter fall at Okinawa was comparable to tropical studies, implying neither lower nor higher reproductive production at the northern limit.

A recent warming trend (BOM, 2018b) could not have initiated colonisation or reproduction at *R. stylosa*'s southern limits, as the most southern mature tree is one of the largest trees south of the Tweed River (710 cm tall in 2005) and large trees and seedlings were present at Corindi Creek in 1980 (Floyd, 1980). However, this does not preclude growth rates and reproductive effort being enhanced by physiologically beneficial small temperature rises.

Reproduction continues at SWR Rocks and at least eight further individuals had recorded away from the mature trees by January 2016 (pers. obs.). It is expected that reproduction will continue at current rates, or with a slight increment from ongoing warming. Monthly maxima since the study (2005–2017) are warm (91% of monthly maxima are above the long-term mean; overall + 0.9 °C). The July monthly minimum has been at the long-term mean or warmer every month (overall + 0.4 °C) (data: BOM, 2018a).

"Precocious" reproduction (*sensu* Dangremond and Feller, 2016) in young *R. stylosa* was not seen during the study, nor during observations in 2010 and 2016. Based on January 2016 observations of marked 2001–2004 seedlings, an approximate minimum time range for initial (very limited) propagule delivery is 12–15 years.

The northern limit of *R. mangle* evidently has greater fluctuation between relatively warm winters and severe impactful cold episodes than at, and beyond, *R. stylosa*'s southern limit, where freezes on the immediate mangrove shore are unknown. Reproductive "anomalies" such as early or exaggerated fruiting may reflect locality- and taxon-specific factors. Perhaps *R. mangle* at its northern limits is closer to its physiological limit than *R. stylosa* is in NSW and is thus more responsive to climatic changes.

5. Conclusion

There was a tendency for SWR Creek to be lower on some measures, such as bud to propagule conversion and some [Organ]-Date and OSI indices, but generally this was due to one only tree there (a large, slowly expanding tree on waterlogged substrate). This, plus intra-estuary variation between trees on numerous parameters, argues against strong latitudinal impact on reproduction over the last 265 km of the range. The short phenological bud and flower phases were tight and peak propagule fall was regional. Endogenous or tree-level exogenous influences on reproduction must be important. There was little evidence of anomalous reproductive responses, although high bud conversion is interesting.

While there must be some link between temperature, plant growth and reproduction, and there is a correlation between temperature (latitude) and mangrove species diversity on the entire Australian east coast, the southern limit of *R. stylosa* appears not to be set physiologically, either reproductively or vegetatively (Wilson and Saintilan, 2012). This suggests caution in generalising about mangrove species responses at the limits under climate change scenarios. Non-physiological factors such as dispersal limitation need to be considered.

Declarations of interest

None.

Acknowledgements and Declaration

NCW was supported by an APA (I) postgraduate scholarship from the Australian Commonwealth Government at ACU. A Strategic Partnership with Industry for Research and Training (SPIRT) Grant to ACU from the NSW Department of Land and Water Conservation provided funding, but no control over findings. Part of the research was undertaken when NCW was at the School of Environmental Science and Management, Southern Cross University in Lismore, NSW in a visiting capacity. Dr Sahadev Sharma provided helpful information on *R. stylosa* on Okinawa. Comments and suggested edits by the anonymous reviewers were very useful.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aquabot.2018.07.009.

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